Molecular and developmental analysis of the fruit abscission zone and shedding process in the oil palm species *Elaeis guineensis* and *Elaeis oleifera*.

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Introduction

Oil palm (*Elaeis guineensis*) belongs to the Arecaceae family and is the number one source of edible vegetable oil worldwide. Previous studies suggest that the fruit shedding process in oil palm is different at the anatomical level from other fruits (Henderson and Osborne, 1990 and 1994; Osborne *et al.*, 1994). In most fruits, there is a synchronized series of cell separations between the fruit and the stalk that leads to fruit shedding. The shedding of the oil palm fruit depends on cell separation events in a primary abscission zone (AZ) and in adjacent zones near the ring of rudimentary stamens (staminodes) and the tepals, with a delay of 1-2 days between each events (Figure 1; Henderson and Osborne, 1990 and 1994; Osborne *et al.*, 1994). We have recently initiated a project with the goal of understanding oil palm fruit shedding at the histological, physiological and molecular levels. In the present study we examined the abscission zones and physiological factors that affect the cell separation processes that lead to fruit shedding in two oil palm species, *Elaeis guineensis* and *Elaeis oleifera*. In addition, we also examined the shedding process in the mantled phenotype of *Elaeis guineensis* in which the staminodes are transformed into carpel-like structures (Adam *et al.*, 2005 and 2007). We have identified a number of gene candidates encoding cell-wall modifying enzymes and have examined their transcript accumulation patterns in the AZ of *E. guineensis*. Our results indicate that the fruit shedding process differs between the two species and will provide an excellent model for understanding the molecular mechanisms associated with abscission and fruit shedding, a key agronomic character of oil palm.

E. guineensis Fruit Abscission Zones

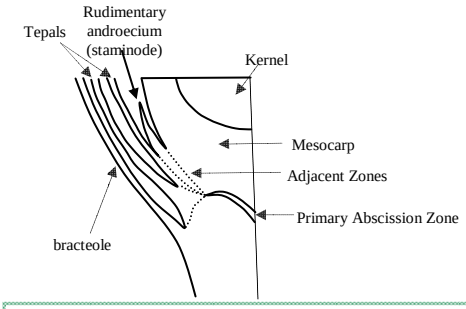


Figure 1. Diagrammatic representation of a partial longitudinal section through an *E. guineensis* fruit to illustrate the primary and adjacent abscission zones (adapted from Osborne *et al.*, 1992).

Inter- and Intra-Species Differences in the Effect of Ethylene on Oil Palm Fruit Abscission

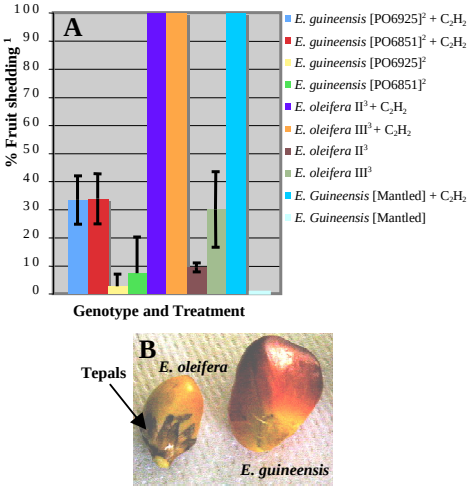


Figure 2. (A) Treatment of spikelets of oil palm fruits with 10^{-5} ppm ethylene for 24h increases the number of fruit that begin to detach[1] from both oil palm species and the mantled fruit phenotype. However, while 100% of the fruit from both the mantled and *E. oleifera* began to be or were completely detached, only 30% of the normal *E. guineensis* fruit began to detach and remained attached at the adjacent zones. **(B)** In addition, *E. oleifera* fruit tepals remained attached to fruit, whereas the *E. guineensis* tepals remain attached to the spikelets after the fruit is shed.

[1]Shedding was determined by applying physical pressure to the fruit to test for the beginning of cell separation events. [2]160 days after pollination (DAP). [3]pollination date unknown, (stage II, green; stage III orange). PO6851 and PO6925 are different *E. guineensis* lines from the same parents.

Ethylene Released During Oil Palm Fruit Development

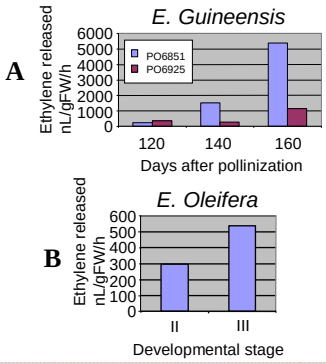


Figure 3. An analysis of the ethylene released during 24 hours from fruits of *E. guineensis* **(A)** and *E. oleifera* **(B)** at different stages of development. While the pollination dates of *E. oleifera* are unknown, stage II is green whereas stage III is orange indicating a more advanced stage of development.

Histological analysis of the oil palm abscission zone

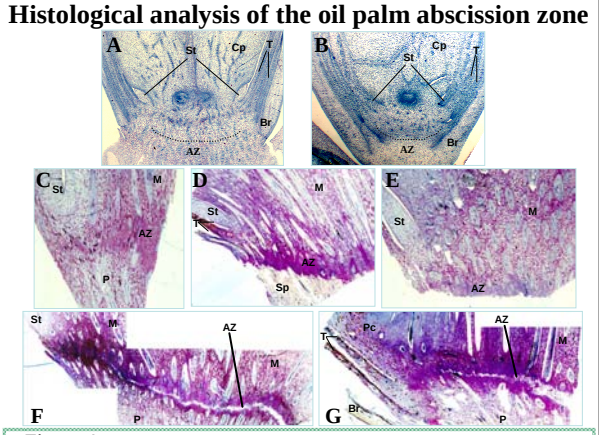
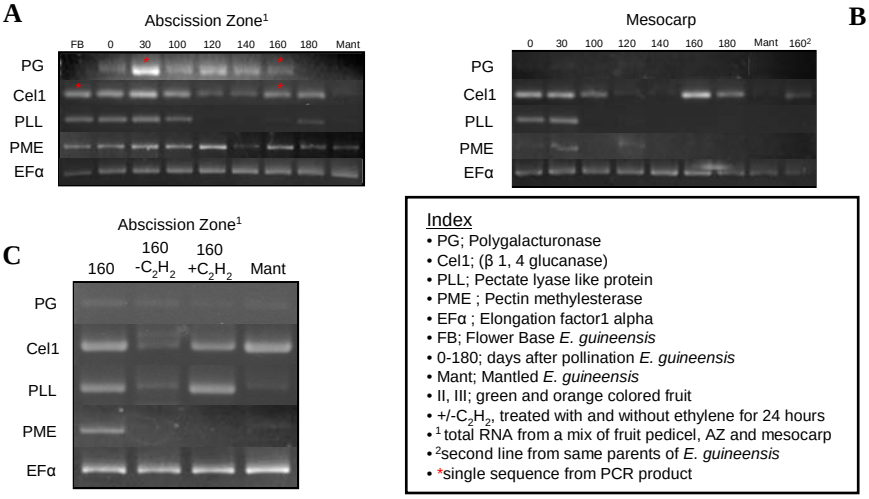


Figure 4. Naphthol Blue Black stained longitudinal sections through abscission zones from oil palm flowers and fruits. **(A)** *E. guineensis* normal **(B)** and mantled female flowers before pollination **(C)** and *E. guineensis* 120-DAP fruit **(D)**; *E. oliefera* fruit before **(E)**, and after C_2H_2 treatment, and **(F)** *E. guineensis* 120-DAP and **(G)**, mantled fruit after C_2H_2 treatment. Above dotted lines indicates the **AZ** (Primary Abscission Zone) in the female flower, **St** (Staminode), **Cp** (Carpel), **Br** (Bracteole), **P** (Pedicel), **T** (Tepals), **Pc** (Pseudocarpel), **M** (Mesocarp).

Cell Wall Modifying Enzymes as Gene Candidates Involved in Cell Separation



Index
- PG; Polygalacturonase
- Cel1; (β 1, 4 glucanase)
- PLL; Pectate lyase like protein
- PME ; Pectin methylesterase
- EFα ; Elongation factor1 alpha
- FB; Flower Base *E. guineensis*
- 0-180; days after pollination *E. guineensis*
- Mant; Mantled *E. guineensis*
- II, III; green and orange colored fruit
- +/-C_2H_2, treated with and without ethylene for 24 hours
- [1]total RNA from a mix of fruit pedicel, AZ and mesocarp
- [2]second line from same parents of *E. guineensis*
- *single sequence from PCR product

Figure 5. (A) Transcript profiles of cell wall modifying gene candidates from *E. guineensis* samples collected from the field were examined in the AZ (A) and mesocarp tissue **(B)** during *E. guineensis* fruit development and in response to ethylene treatment in the laboratory **(C)**. Primers for PG, Cel1, PLL and PME amplified a single band. A PG transcript preferentially accumulated in the AZ samples with the highest accumulation at 30-DAP when compared to the mesocarp of *E. guineensis*. No PG transcript was detected in the mantled samples. Cel1 is found in both the AZ and the mesocarp but appears to be developmentally regulated with lower accumulation at 120 and 140-DAP and is not detected in the mantled fruit. PLL is developmentally regulated with accumulation in both the AZ and the mesocarp in early DAP samples and again in the AZ at 180-DAP. A PME transcript was detected in the AZ at all stages and in the mantled AZ, but was only detected in the 0, 30 and 120-DAP mesocarp samples. Both Cel1 and PLL transcript accumulation is enhanced in the ethylene treated samples, whereas no increase was seen for PG or PME. The PME transcript accumulation was lower in the samples used in the ethylene experiments and the mantled fruit samples than in field collected samples.

Conclusions and Prospects

Here we present the first comparison between the fruit shedding process and abscission zones from the two species of oil palm, *E. guineensis* and *E. oleifera* and the mantled phenotype. Our results indicate that a 24 hour treatment with ethylene induces cell separation to begin in all fruits examined (Figure 2A and 4E, F, G). However, there appear to be differences in the abscission process between the normal *E. guineensis* and *E. oleifera* and the mantled phenotype given their different responses to ethylene. This differential response may be due to differences in the structure of the AZ in the three fruit types or other yet unknown molecular factors. Earlier work indicated that the abscission process in *E. guineensis* is a two-phase delayed process involving primary and adjacent abscission zones (Figure 1; Henderson *et al.*, 1990). When treated with ethylene, *E. oleifera* fruit reveal two characteristics that are different from *E. guineensis*: (1) all the fruit are shed from the spikelet after the 24 hour treatment and (2) do not remained attached at adjacent zones. There are two possible explanations, either there are no adjacent abscission zones in *E. oleifera* and the abscission process has only one phase, or there are adjacent zones but the process could occur within the 24 hour treatment indicating differences in the signalling leading to abscission. In support of the later hypothesis, we found that *E. oleifera* fruit release quantitatively less ethylene during development which suggests differences in the signalling process that leads to abscission. In support for the former hypothesis, histological analysis indicated that the AZ in *E. oleifera* is different from *E. guineensis* given that *E. oleifera* has no pedicel with the fruit attached directly to the spikelet tissue (Figure 4D and E). In addition, the tepals remained attached to the *E. oleifera* fruit that suggests differences in the structure of the AZ in this species. 100% of the mantled fruit begin to shed in response to ethylene, however, some fruit remain attached at adjacent zones which indicates that they still function despite the transformation of the staminode into pseudocarpel near the adjacent zones (Figure 4B and G; Adam *et al.*, 2005,2007). One explanation for this increase in ethylene sensitivity may be due to other modifications that occur in the mantled fruit that effect the abscission process. Finally, towards understanding the molecular mechanisms involved in the abscission process of oil palm, we have identified putative cell wall modifying enzyme genes, including PG, Cel1, PLL and PME from *E. guineensis*. Our results indicate that a transcript for both PG and PME accumulate preferentially in the AZ. In addition, PLL accumulates at 180-DAP when the abscission process may begin to occur in the field. In addition, both the Cel1 and PLL transcripts are enhanced in response to the ethylene treatment that leads to cell separation and fruit shedding (Figures 1, 2 and 5). In the future, we plan a comprehensive histological analysis of *E. guineensis*, *E. oleifera* and the mantled phenotype, to clone cell wall modifying genes from *E. guineensis* and in addition, a pyrosequencing analysis of the AZ transcriptome of *E. guineensis*.

References

Henderson J, Osborne DJ (1990) Cell Separation and Anatomy of Abscission in the Oil Palm, *Elaeis guineensis* Jacq. J Exp Bot 41: 203-210.
Osborne DJ, Henderson J, Corley RHV (1992) Controlling fruit-shedding in the oil palm. Endeavour 16: 173-177.
Henderson J, Osborne DJ (1994) Intertissue Signaling during the 2-Phase Abscission in Oil Palm Fruit. Journal of Experimental Botany 45: 943-951.
Henderson J, Davies HA, Heyes SJ, Osborne DJ (2001) The study of a monocotyledon abscission zone using microscopic, chemical, enzymatic and solid state C-13 CP/MAS NMR analyses. Phytochemistry 56: 131-139.
Adam H, Jouannic S, Escoute J, Duval Y, Verdeil JL, Tregear JW (2005) Reproductive developmental complexity in the African oil palm (*Elaeis guineensis*, Arecaceae). American Journal of Botany 92: 1836-1852.
Adam H, Jouannic S, Orieux Y, Morcillo F, Richaud F, Duval Y, Tregear JW (2007) Functional characterization of MADS box genes involved in the determination of oil palm flower structure. J Exp Bot 58: 1245-1259

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